

2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) as a Potent and Persistent Thyroxine Agonist: A Mechanistic Model for Toxicity Based on Molecular Reactivity

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TCDD and thyroxine have common molecular reactivity properties which enable them to present a planar face and lateral halogens in interactions with proteins. These molecular properties are consistent with the structure-toxicity relationship for TCDD and related compounds. Biological evidence is discussed including preliminary studies on the effects of TCDD exposure on tadpole growth and development which is consistent with the possible thyroxine-like activity of TCDD. The work suggests the possibility that toxicity is at least in part the expression of potent and persistent thyroid hormone activity (responses induced by TCDD which qualitatively correspond to those mediated by thyroid hormones). A mechanism for toxicity is proposed which involves receptor proteins; the planar aromatic system controls binding to cytosolic proteins and halogen substituents regulate binding to nuclear proteins. This simple model based on molecular reactivity sheds light on the diversified effects of TCDD and related compound toxicity and on certain thyroid hormone action. The model also permits predictions to be made with regard to the toxicity and thyroid hormone activity of untested compounds. In addition, the model suggests a general mechanism for hormone action based on metabolically regulated differential and cooperative protein receptor binding events in cellular compartments which can explain agonism, antagonism and potentiation within the framework of receptor occupancy theory.

Introduction

The halogenated aromatic hydrocarbons constitute a broad class of environmentally important compounds with varying structure and toxicity (1). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (dioxin or TCDD) the prototypical structure (Fig. 1) is the most toxic compound of this type and is also a potent inducer of cytochrome P-448-mediated mixed-function oxidase enzyme systems. The mechanism of toxicity of these compounds has been under intense study in a number of laboratories throughout the world. The toxicity and induction responses have both been proposed to involve initial binding of the hydrocarbon to the same cytosolic (*Ah* or dioxin receptor)

receptor, but the subsequent events are not completely understood (2). We recently provided evidence in the form of theoretical and experimental models that the structure-toxicity (defined as the acute lethal toxicity normally associated with TCDD) and the structure-induction relationships are different. Binding to the induction receptor appears to involve the aromatic rings in a stacking type interaction while binding to the hypothetical "toxic" receptor may involve polarizability interactions with halogen substituents (3). To account for the differences in the structure-activity relationships, a cooperative receptor (requiring interaction of TCDD with two proteins with different structural parts) mechanism for toxicity was postulated. This work also suggested that a more toxicologically relevant binding protein should be sought because of the requirements of planarity and halogenation for toxicity, as opposed to induction.

The thyroid hormone-binding proteins are likely candidates as receptors for TCDD and related compounds because of their specificities for binding the biologically

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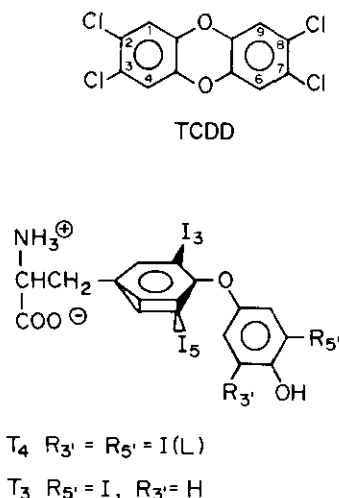


FIGURE 1. TCDD and thyroid hormone structures.

relevant halogenated aromatic hydrocarbon hormones, thyroxine (T₄) and triiodothyronine (T₃) (Fig. 1). Thyroxine-binding prealbumin (TBPA) is a major carrier protein for T₄ in blood and has been used by others as a model for the interaction of thyroid hormones with the nuclear thyroid hormone receptor (4). Using interactive computer graphics, the molecular interactions between TCDD and TBPA were modeled. This work succeeded in linking the structure of TCDD to the structure of T₄. We were also able to demonstrate that TCDD type structures could compete with T₄ for binding to TBPA (5). This nuclear receptor model for thyroxine fulfills the requirements of the hypothetical "toxic" receptor since it requires halogenation in lateral positions for effective binding. The structural resemblance of certain chlorinated aromatic hydrocarbons to the thyroid hormones and the potential of these compounds to act as thyroid hormone agonists or antagonists and produce aberrant thyroid activity was first recognized over 15 years ago (6).

Thus a plausible mechanism for TCDD and related compound toxicity could be associated with their ability specifically to bind proteins and to function as potent and persistent thyroxine agonists or antagonists. Consistent with this suggestion is a range of clinical and biochemical findings in TCDD treated animals that resemble both the hypo- and hyperthyroid disease states (2,7). Serum T₄ depletion has been a consistent finding along with unchanged or slightly elevated triiodothyronine (T₃) levels (8,9), suggesting perhaps more direct involvement of T₄ specific binding proteins and receptors. The long-range objectives of this work were to identify common molecular reactivity properties for TCDD and T₄ consistent with the structure-toxicity relationships; to propose a simple mechanistic model for toxicity based on molecular structure and reactivity that could explain the structure-toxicity results and is consistent with major clinical and toxicological outcomes of these and related compounds and has predictive value for untested compounds, and to establish that TCDD is

a thyroxine agonist at the level of tissue receptors and link the mechanism of toxicity to the mechanism of thyroid hormone action.

Experimental

Materials

The TCDD used in this work was synthesized at this institute according to published procedures (10). Gas chromatography-mass spectroscopy was used to determine purity which was >99%.

The ¹²⁵I-thyroxine (~1250 µCi/µg) used in this work was obtained commercially (New England Nuclear Corp). The radiochemical purity determined by the manufacturer approximately one month prior to use by an HPLC method was > 99%. Radiochromatography performed in our laboratory 24 hrs prior to use, using a TLC silica gel strip and a developing solvent composed of hexane:2-methyl-2-butanol:methanol:NH₄OH (4N), 1:6:2:1, revealed a radiochemical purity > 97%.

The labeled hormone is shipped in 50% *n*-propanol which is unsuitable for parenteral use in animals. Thus, immediately before dosing (by gavage) the hydroalcoholic solution of ¹²⁵I-T₄ was rapidly reduced in volume by a nitrogen stream until the original volume of 2.5 mL had been reduced to 1.6 mL. This solution was brought to 2.0 mL total volume by adding 0.4 mL of sodium chloride solution, 0.9%, USP. Each animal received 0.3 mL (80 µCi or 88 ng) of this solution.

Molecular Modeling

The model building shown in Figure 3 was carried out in the computer graphics laboratory of the Department of Chemistry, University of California, San Diego, using a Silcon Graphics Iris system. The coordinates for TCDD and T₄ were those previously used (5) with some small adjustments for the torsion angles about the ether linkage of the T₄ structure. A skewed conformation with torsion angles $\phi/\phi' = 80^\circ/0^\circ$ was used which is well within the acceptable range as indicated by energy calculations (11) and crystallographic observations (12) on a variety of similar structures. The overall relationship of the two molecules was modeled as suggested by our previous work (5) with prealbumin where there is close correspondence of the lateral chlorines in TCDD with the lateral iodines and hydroxyl group in the phenolic ring of T₄. The superposition stereopair model shown (Fig. 3) represents the least-squares fit on the important atoms. Table 1 shows the atomic deviations for the important atoms in this fit.

Thyroxine Measurements

Serum: Rats/Guinea Pigs. Groups of five male Sprague-Dawley CD rats (200-250 g, 6 weeks old) were administered TCDD at 0, 12, 25, 37, or 50 µg/kg body weight doses. Each animal was given TCDD in corn oil

Table 1. Least-squares fit of TCDD and T₄ structures.

| TCDD ^a | T ₄ ^a | Atomic deviations, Å |
|-------------------|------------------------------|----------------------|
| Cl-3 | CH ₂ (side chain) | 0.12 |
| O-10 | O (ether) | 0.86 |
| O-5 | I-5 | 2.89 |
| Cl-7 | O (hydroxyl) | 1.02 |
| Cl-8 | I-5' | 0.81 |
| C-1 | I-3 | 1.95 |
| C-6 | I-3' | 1.81 |

^aSee Fig. 1 for location of atoms.

(0.1 mL/kg body weight) by gavage and housed and maintained according to standard procedures (10). Control animals were dosed similarly with corn oil alone. Groups of five male Hartley strain guinea pigs (100–200 g, 3.5 weeks old) were similarly dosed with TCDD at 0, 0.5, 1.0, 1.5, or 2.0 µg/kg body weight. All animals were held 9 days before sacrificing with CO₂ euthanasia. Blood was taken by cardiac puncture after breathing had stopped. Blood was allowed to clot, spun down, and the serum transferred to another tube and spun again before use.

Serum thyroxine measurements were made by using a standard radioimmunoassay kit from commercial sources (Tetra-Tab RIA, Nuclear-Medical Laboratories, Irving, TX). In this procedure an acid reagent is mixed with a serum sample to release the thyroxine from proteins. The thyroxine is then allowed to compete with radiolabeled thyroxine for a limited number of binding sites on highly specific antibody. Free and antibody-bound fractions are then separated by addition of an ammonium sulfate solution to precipitate the antibody-bound fraction. Following centrifugation, supernatant fluid is discarded and the radioactivity in the precipitate is measured directly in a gamma scintillation spectrometer. Results are determined by comparison with a standard curve.

Tissue: Rat. Groups of three male Sprague-Dawley CD rats (200–250 g, 6 weeks old) were administered TCDD at 0 and 25 µg/kg body weight doses. Each animal was given TCDD in corn oil by gavage (0.1 mL/kg body weight) and housed and maintained according to standard procedures (10). Control animals were dosed with corn oil alone. All animals were held for 9 days before receiving 0.3 mL of ¹²⁵I-thyroxine solution by gavage, equivalent to 80 µCi and 88 ng of the labeled hormone. Immediately following this treatment, each animal was anesthetized by using metofane, and whole body radioactivity was determined (day 0) by using a computer controlled gamma camera with a pin-hole collimator with careful and fixed positioning of the animal. These measurements were again made on each animal 24 and 48 hr later (day 1 and day 2). After the day 2 measurements were made the animals were sacrificed by euthanasia. Blood was taken by cardiac puncture after breathing had stopped. Organs were surgically removed and placed into tared vials for weighing and gamma counting.

Tadpole Study

Materials. Tadpoles of *Xenopus laevis* were obtained commercially from Nasco at stages 48 to 55 (Nasco Flyer No. 649) in which total length ranged from 14 to 80 mm. They were allowed to reach room temperature overnight then mixed with an equal volume of chlorine-free water (well water) at the same temperature. Experimental animals were visually sorted into sizes ranging from 27 to 37 mm (stage 51), an interval when hind leg development was approximately 1 to 2 mm. Measurement parameters were obtained from serial photographs. Twenty tadpoles in 150 mL of water were added to each aquarium. Frog brittle from Nasco, a special food formulation for *Xenopus* tadpoles, was blended for 1 min (2 g powder/100 mL deionized water). The suspension was maintained by successive drawing and expelling in a 10 mL syringe, and 5 mL was injected into the water (1 gal). Suspension volumes were further adjusted as needed so that the water became clear within 24 hr. Fresh suspensions were prepared daily.

Aquarium water was changed at intervals not exceeding 8 days. To minimize animal injury, tadpoles were removed by scooping rather than collecting in nets. Before returning to a fresh water change, they were photographed in a 7 × 7 × 3 in. transparent plastic container (made of acrylic and glued together with methylene chloride) on translucent background illumination. Since the camera was hand-held, a transparent grid of 10 squares/in, 7 in. long was included in the photo so that size corrections could be made between different photographs. Tadpoles were identified as frogs when both sets of legs were fully functional and the body structure was that of a frog.

Aquarium Maintenance Because of the toxicity of TCDD, 2.5 gal. glass-covered aquarium tanks were placed into standard plastic rat cages to collect any accidentally spilled aquarium contents. Well water was collected in 1-gallon, screw-cap glass jugs 1 or 2 days before use. Just before use, 0.8 mL acetone was added without mixing, followed with another 0.8 mL acetone containing 4 µg, 2 µg, and 0.4 µg TCDD, respectively (approximately 1.0, 0.5, and 0.1 ppb). Control water contained 1.6 mL acetone only. Mixing was by inversion 10 to 12 times. Before placing tadpoles in the tank, food suspension was immediately added. Water was aerated with an air pump by bubbling through an airstone diffuser. Acetone (0.2 mL) was added daily directly over the diffuser to maximize mixing and maintain solubility of TCDD. Liquid crystal thermometers on the outside of the jugs and aquaria were used to monitor water temperature kept at 68 ± 2°F.

Logistics of Handling Used TCDD Water and the Cleaning of Aquarium Tanks. When tank water was to be changed the animals were first removed and then the aquaria were emptied through funnels into 4-L Erlenmeyer flasks. The solution was vacuum-filtered once to remove particulate matter through a Whatman 15 cm No. 651 and CD/F microcrystalline glass filters con-

Table 2. Whole body counts from TCDD-treated rats receiving ^{125}I -thyroxine 9 days later.^a

| Time, days | Control | | Treated | |
|----------------|------------------|---------------------|------------------|---------------------|
| | Animal | Counts | Animal | Counts |
| 0 ^b | A | 2540 | X | 4340 |
| | B | 2940 | Y | 4140 |
| | C | 3140 | Z | 3640 |
| | Mean (\pm SD) | 2873 (\pm 305.5) | Mean (\pm SD) | 4040 (\pm 360.6) |
| | | | | |
| 1 | A | 1040 | X | 940 |
| | B | 1040 | Y | 520 |
| | C | 1040 | Z | 840 |
| | Mean (\pm SD) | 1040 (\pm 0) | Mean (\pm SD) | 767 (\pm 219.4) |
| | | | | |
| 2 | A | 430 | X | 240 |
| | B | 440 | Y | 120 |
| | C | 510 | Z | 300 |
| | Mean (\pm SD) | 460 (\pm 43.6) | Mean (\pm SD) | 220 (\pm 91.7) |
| | | | | |

^aObtained using gamma camera with a pen-hole collimator with careful and fixed positioning of the animals and background corrected.

^bDay 0 is immediately following (\sim 0.5 hr later) the ^{125}I -thyroxine treatment.

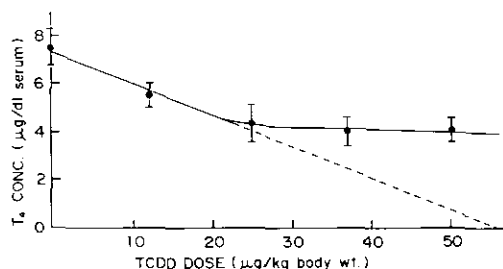


FIGURE 2. Thyroxine concentration in rat serum as a function of TCDD dose (mean \pm SD, $N = 5$).

Table 3. Tissue counts from TCDD-treated rats receiving ^{125}I -thyroxine 9 days later and held for another 2 days.

| Tissue | Control (mean \pm SD) ^a | Treated (mean \pm SD) ^a | Control Treated |
|----------|---|---|--------------------|
| Liver | 82,471 (\pm 3414) | 15,791 (\pm 7146) | 5.22 |
| Blood | 70,883 (\pm 7297) ^b | 18,407 (\pm 8837) | 3.85 |
| Spleen | 20,002 (\pm 2104) | 5,333 (\pm 1880) | 3.75 |
| Kidney | 70,277 (\pm 5872) | 20,163 (\pm 8219) | 3.49 |
| Adrenal | 42,113 (\pm 8880) | 12,887 (\pm 5348) | 3.27 |
| Thymus | 14,644 (\pm 2546) | 6,063 (\pm 2451) | 2.42 |
| Thyroid | 28,682,834 (\pm 9,016,564) | 18,572,556 (\pm 6,158,614) | 1.54 |
| GI tract | 86,308 (\pm 39,541) | 113,718 (\pm 42,717) | 0.76 |

^a $n = 3$ unless specified otherwise.

^b $n = 2$.

tained in a Buchner funnel. Fresh filter papers were again added plus a mixture of activated charcoal:Celite (1:1 w/w) for final filtration. Filtrates were stored in 40-L Nalgene screw-cap carboys to be later discarded.

Before refilling the aquaria, tank sides and bottom were first wiped clean with moistened paper towels followed with acetone-soaked towels to remove residual TCDD. To minimize chance infection from aeromonas bacteria, cleansing powder was used to wipe sides and bottom. After thorough tap water rinsing, the tank was towel dried. The paper products were bagged and incinerated.

Statistical Methods

Linear regression analyses (13) were employed to determine whether or not tadpole length, width or size were age-related in dosed and control groups. Analysis of variance procedures (14) were used to assess the significance of dose and age-effects. Two-sided *t*-tests (14) were used to make pairwise comparisons between dosed and control groups. Probabilities of frog formation and rates of death were estimated by the product limit procedure of Kaplan and Meier (15).

Results

Serum T4 levels were determined in rats following single oral doses of 12, 25, 37, and 50 $\mu\text{g}/\text{kg}$ body weight

of TCDD given 9 days earlier. The results of these measurements are shown in Figure 2. T4 levels appeared to be linearly depleted between 0 and 25 $\mu\text{g}/\text{kg}$ doses and remained fairly constant at about half the control level at the higher more toxic doses. Extrapolation of the linear phase to the dose for complete depletion of serum T4 corresponds closely with the lethal dose of TCDD in this rat species. A similar study in a much more TCDD sensitive guinea pig with TCDD dose levels between 0 and 2 $\mu\text{g}/\text{kg}$ body weight afforded serum T4 levels in control animals ($2.78 \pm 0.57 \mu\text{g}/\text{dL}$, $n = 5$) about one-third the level found in rats and T4 levels in TCDD-treated animals consistently elevated by about 1 $\mu\text{g}/\text{dL}$ (complete data not shown).

Studies of TCDD-treated rats (25 $\mu\text{g}/\text{kg}$ body weight) given a tracer dose of ^{125}I -T4 9 days later showed that the TCDD-treated animals excreted T4 at about twice the rate as controls (Table 2), and this is reflected in depletion of tissue T4 as indicated in Table 3. This pattern was reversed in the GI tract as expected, since this tissue is involved in the elimination of T4 glucuronide.

In order to test the possibility that TCDD can function as a thyroid hormone agonist and interact directly with tissue receptors, the effects of TCDD on tadpole growth and development were studied. For reasons of safety and convenience, the effects were measured

Table 4. Average growth parameters of *Xenopus laevis* tadpoles as a function of age and TCDD concentration in water.

| Group ^a | No. | | Age, days | Size parameter ^c | | |
|--------------------|----------------|----------------|-----------------|-----------------------------|--------------|-------------------|
| | T ^b | F ^b | | Length, in. | Width, in. | Size ^d |
| C | 20 | 0 | 8 | 0.49 ± 0.06 | 0.26 ± 0.04 | 0.130 ± 0.030 |
| Lo | 20 | 0 | 8 | 0.55 ± 0.07* | 0.26 ± 0.05 | 0.147 ± 0.041 |
| M | 20 | 0 | 8 | 0.43 ± 0.05* | 0.22 ± 0.05* | 0.095 ± 0.028* |
| H | 20 | 0 | 8 | 0.36 ± 0.06* | 0.19 ± 0.05* | 0.70 ± 0.18* |
| C | 20 | 0 | 16 | 0.47 ± 0.07 | 0.28 ± 0.04 | 0.135 ± 0.038 |
| Lo | 20 | 0 | 16 | 0.53 ± 0.08* | 0.28 ± 0.06 | 0.151 ± 0.049 |
| M | 7 | 0 | 16 | 0.41 ± 0.06* | 0.18 ± 0.03* | 0.075 ± 0.020* |
| H | 0 | 0 | 16 | — | — | — |
| C | 20 | 0 | 23 | 0.52 ± 0.08 | 0.28 ± 0.05 | 0.154 ± 0.039 |
| Lo | 18 | 1 | 23 | 0.49 ± 0.08 | 0.27 ± 0.04 | 0.130 ± 0.040 |
| M | 0 | 0 | 23 | — | — | — |
| H | 0 | 0 | 23 | — | — | — |
| C | 19 | 0 | 30 ^e | 0.53 ± 0.06 | 0.27 ± 0.05 | 0.145 ± 0.035 |
| Lo | f | f | 30 ^e | f | f | f |
| M | 0 | 0 | 30 | — | — | — |
| H | 0 | 0 | 30 | — | — | — |
| C | 17 | 2 | 38 | 0.49 ± 0.07 | 0.27 ± 0.04 | 0.135 ± 0.037 |
| Lo | 13 | 3 | 38 | 0.45 ± 0.07 | 0.24 ± 0.04 | 0.112 ± 0.030 |
| M | 0 | 0 | 38 | — | — | — |
| H | 0 | 0 | 38 | — | — | — |
| C | 16 | 3 | 42 | 0.48 ± 0.06 | 0.26 ± 0.05 | 0.128 ± 0.038 |
| Lo | 9 | 2 | 42 | 0.43 ± 0.04 | 0.24 ± 0.04 | 0.106 ± 0.024 |
| M | 0 | 0 | 42 | — | — | — |
| H | 0 | 0 | 42 | — | — | — |
| C | 13 | 1 | 51 | 0.51 ± 0.05 | 0.28 ± 0.05 | 0.144 ± 0.041 |
| Lo | 8 | 2 | 51 | 0.42 ± 0.05* | 0.23 ± 0.04 | 0.098 ± 0.026* |
| M | 0 | 0 | 51 | — | — | — |
| H | 0 | 0 | 51 | — | — | — |
| C | 10 | 3 | 58 | 0.50 ± 0.07 | 0.28 ± 0.07 | 0.146 ± 0.055 |
| Lo | 1 | 0 | 58 | — | — | — |
| M | 0 | 0 | 58 | — | — | — |
| H | 0 | 0 | 58 | — | — | — |
| C | 5 | 3 | 66 | 0.45 ± 0.04 | 0.25 ± 0.03 | 0.115 ± 0.019 |
| Lo | 1 | 0 | 66 | — | — | — |
| M | 0 | 0 | 66 | — | — | — |
| H | 0 | 0 | 66 | — | — | — |

^aGroup: C = control; Lo = low dose (0.1 ppb); M = medium dose (0.5 ppb) H = high dose (1.0 ppb).^bT = tadpoles; F = frogs (total number of living animals).^cLength and width parameters of each tadpole were measured directly from photographs which were corrected from a grid (10 lines/inch) included in the photo. Length measurements included the body but not the tail. Widths were measured at the widest point just behind the eyes. Values are means ± SD (in inches).^dS = size (length × width).^eTadpoles were transferred to TCDD-free water.^fPhoto not available.**p* < 0.05 relative to controls.

through the use of serial photographs. Tadpoles receiving medium doses (0.5 ppb) and high doses (1.0 ppb) of TCDD showed significant dose-related decreases in length from controls (*p* < 0.05) within 8 days after the beginning of the experiment (Table 4). All tadpoles were dead by day 16 and day 23, respectively, for the high and medium dose levels. Even by day 16 the 0.5 ppb dose level mortality was 65%. Similar results were obtained in earlier studies (data not shown).

Tadpoles dosed at the lower level (0.1 ppb), on the

other hand, showed significantly greater growth than the control by day 8 and 16 for length (*p* < 0.05). However, after day 16 growth values were less than the controls but the difference was not significant until day 51 (*p* < 0.02 in length and size only). Tadpoles dosed at the 0.1 ppb level were maintained in TCDD-free water from day 30 to the end of the experiment to avoid the possibility that all tadpoles might die before being transformed to frogs.

The results of linear regression analysis indicated that

for the controls there was no significant change in length, width, or size over time (Table 5). In contrast, for the low dose (0.1 ppb) groups, all three of these parameters showed time-related decreases.

The number of tadpoles that eventually developed

Table 5. Estimated coefficients of linear regression of tadpole growth over time.

| Group | Variable ^a | Intercepts | Slope | p Value ^b |
|-----------------------|-----------------------|------------|-----------|----------------------|
| Control | Length | 0.50 | -0.00014 | 0.69 |
| | Width | 0.27 | -0.000019 | 0.94 |
| | Size ^c | 0.14 | -0.000034 | 0.86 |
| Low dose (0.1 ppb) | Length | 0.57 | -0.0031 | 0.0001 |
| | Width | 0.28 | -0.00078 | 0.023 |
| | Size ^c | 0.16 | -0.0012 | 0.0001 |

^aUnits of measure: inches for length and width; (inch)² for size.

^bFor testing the hypothesis of zero slope, i.e., is tadpole growth significantly affected over time.

^cLength × width.

into frogs was identical (7) in dosed and control groups. There were, however, differences between the two groups. Not only did frogs appear earlier in the dosed group as compared to the controls, but there was also a tendency for accelerated metamorphosis. Frogs in the dosed group also showed evidence of muscle tremors consistent with hyperactivity. By day 42 seven frogs were formed in the dosed group but only three were produced in the controls. At the end of the experiment there were seven and four dead frogs in the dosed and control groups, respectively. Those in the dosed group also tended to die earlier. A life table analysis (method which corrects for survival differences in groups) found this difference to be significant ($p < 0.05$) (Table 6).

In the dosed group from day 23 to day 41 no tadpole deaths occurred, and six of the seven frogs appeared. On the next day (day 42) three tadpoles died but one new frog was formed. From this time on, no new frogs appeared. Tadpoles on day 51 (see Table 4) showed sta-

Table 6. Estimated Kaplan-Meier (K-M) probabilities for formation and rates of death of frogs in TCDD-contaminated water.

| Dose level, ppb | Days dosed | No. T ^a | No. NF ^a | No. DF ^a | % Cumulative K-M probabilities | |
|--------------------|------------|--------------------|---------------------|---------------------|--------------------------------|------|
| | | | | | NF | DF |
| 0 | 8 | 20 | 0 | 0 | 0 | 0 |
| | 16 | 20 | 0 | 0 | 0 | 0 |
| | 18 | - | - | - | 0 | 0 |
| | 23 | 20 | 0 | 0 | 0 | 0 |
| | 29 | 19 ^b | 0 | 0 | 0 | 0 |
| | 30 | 19 | 0 | 0 | 0 | 0 |
| | 31 | - | - | - | 0 | 0 |
| | 38 | 17 | 2 | 0 | 10.5 | 0 |
| | 41 | - | - | - | 10.5 | 0 |
| | 42 | 16 | 1 | 0 | 15.8 | 0 |
| | 44 | 15 ^b | 0 | 0 | 15.8 | 0 |
| | 45 | 14 ^b | 0 | 1 | 15.8 | 5.9 |
| | 48 | - | - | - | 15.8 | 5.9 |
| | 51 | 13 ^b | 0 | 1 | 15.8 | 11.8 |
| | 52 | - | - | - | 15.8 | 11.8 |
| | 53 | - | - | - | 15.8 | 11.8 |
| | 58 | 10 | 3 | 1 | 35.2 | 18.1 |
| | 66 | 5 ^c | 1 | 1 | 43.3 | 25.5 |
| 0.1 | 8 | 20 | 0 | 0 | 0 | 0 |
| | 16 | 20 | 0 | 0 | 0 | 0 |
| | 18 | 19 ^b | 0 | 0 | 0 | 0 |
| | 23 | 18 | 1 | 0 | 5.3 | 0 |
| | 29 | - | - | - | 5.3 | 0 |
| | 30 | 16 | 2 | 0 | 15.8 | 0 |
| | 31 | 16 | 0 | 3 | 15.8 | 15.8 |
| | 38 | 13 | 3 | 0 | 31.6 | 15.8 |
| | 41 | 13 | 0 | 1 | 31.6 | 21.1 |
| | 42 | 9 ^d | 1 | 1 | 37.3 | 26.7 |
| | 44 | - | - | - | 37.3 | 26.7 |
| | 45 | - | - | - | 37.3 | 26.7 |
| | 48 | 8 ^b | 0 | 0 | 37.3 | 26.7 |
| | 51 | 8 | 0 | 0 | 37.3 | 26.7 |
| | 52 | 8 | 0 | 1 | 37.3 | 34.0 |
| | 53 | 8 | 0 | 1 | 37.3 | 41.4 |
| | 58 | 1 ^e | 0 | 0 | 37.3 | 41.4 |
| | 66 | 1 | 0 | 0 | 37.3 | 41.4 |

^aT = Tadpole; NF = new frogs; DF = dead frogs.

^b1 Tadpole dead.

^c4 Tadpoles dead.

^d3 Tadpoles dead.

^e7 Tadpoles dead.

tistically significant body size diminution from the control group, suggestive of TCDD toxic effects. Also 7 days later (day 58), seven of eight tadpoles were dead. The control group on day 58 had 10 live tadpoles as well as three new frogs. By day 66, four tadpoles were dead but one new frog was formed. The cause of the four tadpole deaths at day 66 in the control group is not known, but a limited number of frogs can be formed while tadpoles are dying. The effect of the daily addition of acetone was not separately studied.

Discussion

Most signs of TCDD (and related compounds) toxicity resemble signs seen in thyroid dysfunction, e.g., loss of body weight and appetite, bradycardia and tachycardia, alopecia and hirsutism, hyperkeratosis, altered levels of triglycerides and cholesterol (2,7). The role of the thyroid in mediating the toxicity of TCDD has been considered (8). TCDD and related compounds have been shown to deplete thyroxine (T4) in a dose-dependent manner (8,9), suggesting perhaps more direct involvement of T4-binding proteins and receptors. The thyroid hormones are water-insoluble molecules that require specific binding proteins in the plasma and the cell cytosol to enable them to gain access to nuclear receptors (16). Thus, a plausible mechanism for TCDD and related compound toxicity could be associated with their ability to bind thyroid hormone receptors and function as potent and persistent agonists or antagonists.

Common Molecular Reactivity Properties of TCDD and T4

If TCDD is replacing T4 at the level of the nuclear receptors, T4 must also possess molecular reactivity characteristics in common with TCDD. TCDD is a relatively planar and rigid structure which can present a planar face and lateral halogens in interactions with proteins. T4 is also conformationally rigid (12) because of the steric constraints of the large iodine atoms in the 3,5 positions and can also present a planar face, although nonplanar in total structure, and lateral halogens in interactions with proteins. Figure 3 shows the stereopair superposition of TCDD and T4. The relationship of the two structures is primarily determined by the correspondence of the lateral halogen substituents as pre-

dicted by the TCDD-prealbumin modeling studies (5) and by a least squares fit of the important atoms (Table 1). In this arrangement, TCDD can best model T4 in terms of overall molecular reactivity. In this modeling exercise it is clear that the part (the planar face) of the molecular structure of T4 that interacts with the *Ah* receptor has to be the nonphenolic ring bearing the alanine side chain (See Fig. 3 and Fig. 4, interaction model, *Rn Dx Ah**). The planar face of the phenolic ring is sterically inaccessible. Molecular size and shape are not controlling factors in binding the *Ah* receptor (3). A better model is based on molecular polarizability (dispersion interactions) and equilibrium separation distance between receptor and effector molecule. This is consistent with a stacking or layering of molecules type model as in charge-transfer complexation. The *Ah* receptor appears to bind T3 with about five to ten times the affinity of T4 but binds TCDD with about 10 times the affinity of T3 (17).

Formation of charge-transfer complexes involving accessible planar faces of these molecules is suggested. Toxic structures related to TCDD are known (3) to form charge transfer complexes with donor molecules and we have recently demonstrated in preliminary studies (data not shown) involving NMR spectroscopic measurements that complexes of this type are also formed with the nonphenolic ring of T4 and T3. Thus TCDD and active hormones appear to have common molecular reactivity properties with regard to their interaction with the *Ah* receptor.

In recent studies of the interactions of compounds related to TCDD with TBPA (5), a model for the nuclear thyroid hormone receptor (4), we demonstrated using computer graphics and competitive binding experiments that dioxin type structures can be effective competitive binding ligands for thyroxine-specific binding sites in prealbumin and that lateral chlorine substitution is important in this interaction. These studies linked the structure of thyroxine to TCDD and established yet another common molecular reactivity property (lateral halogens) of these molecules that is consistent with the structure-toxicity relationship and the requirements of planarity of structure and lateral halogenation in toxicity. Thus, both TCDD and T4 are conformationally rigid structures which have accessible planar faces and lateral halogens and have been shown to bind the same protein models. These protein models require planar structures and lateral halogenation for strong binding and therefore have toxicological relevance.

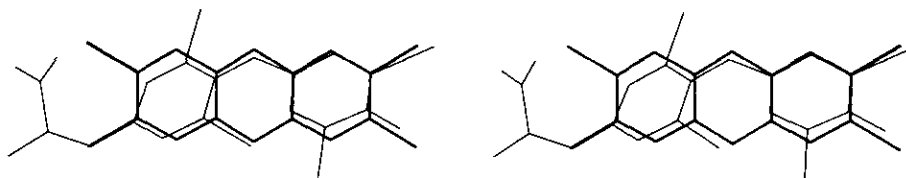


FIGURE 3. Stereopair superposition of TCDD and T4 in cooperative binding model (*Rn Dx Ah**; see Fig. 4).

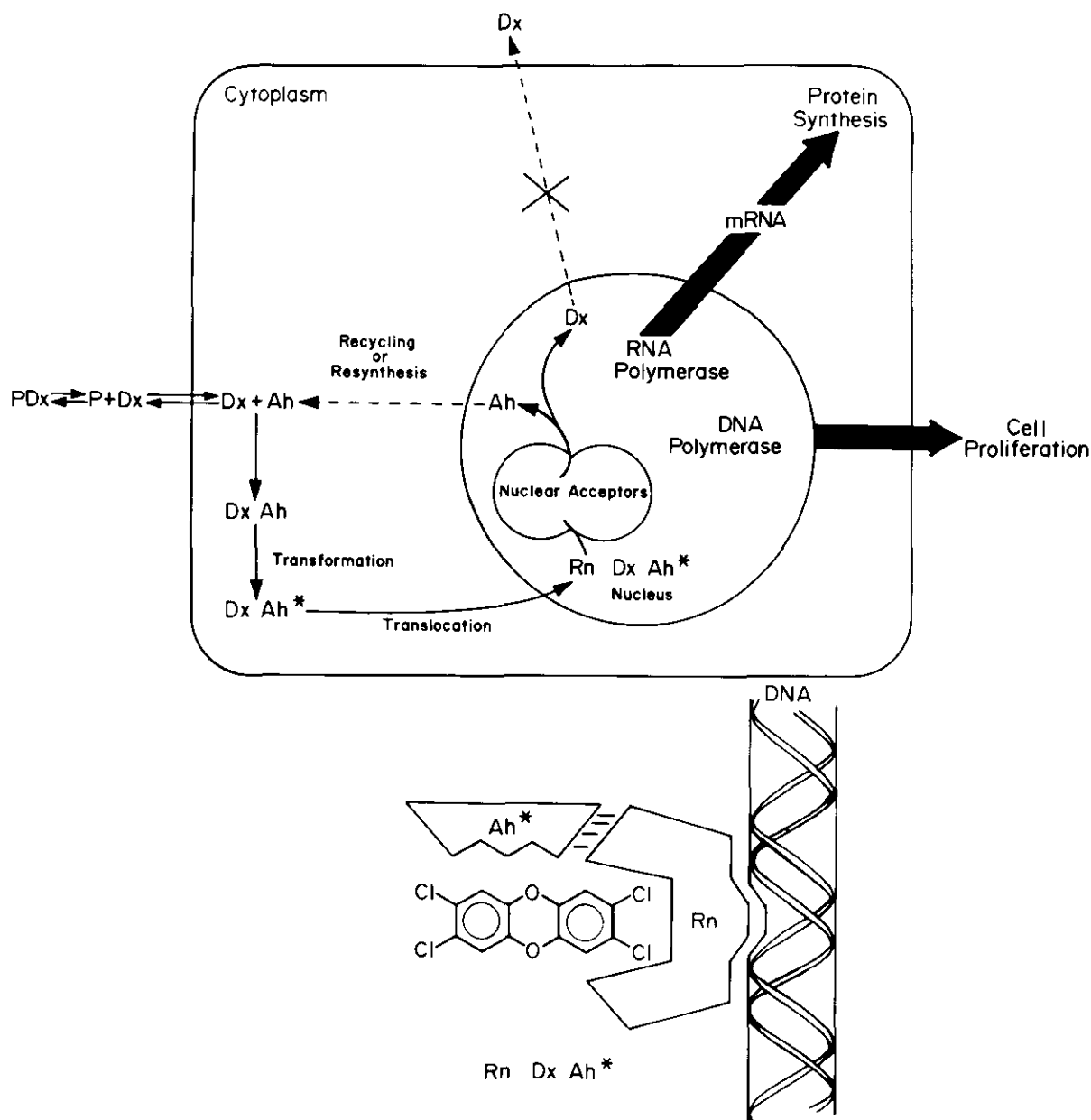


FIGURE 4. Simple mechanistic model for TCDD toxicity based on molecular reactivity properties.

Cooperative Protein Receptor Mechanism Model for Toxicity

In our previous work (5), we proposed a new cooperative protein receptor mechanism for TCDD action based on molecular structure and reactivity considerations involving relevant protein interactions. We now further define this mechanism (see Fig. 4). In this model, the Ah receptor can be viewed as a storage and translocating protein for thyroid hormones which is in equilibrium with the nuclear binding events and under certain conditions can be found in both cytosolic and nuclear compartments of the cell. T3 binds to nuclear binding sites with markedly higher affinity than to cytosolic sites

(18), but the cytosol has greater binding capacity for T3 and presumably TCDD. The somewhat higher affinity of T3 (as compared to T4) for the cytosolic sites may serve to selectively displace T4 toward nuclear sites as T3 accumulates from metabolism of T4. In contrast, TCDD can apparently bind both the cytosolic and nuclear sites with high affinity and for TCDD the cytosolic sites would be sites of loss with respect to the nuclear sites. The equilibrium movement of these storage and translocating proteins in and out of the nucleus can thus be controlled by a concentration gradient determined by available binding ligands and their affinities and protein binding capacity. The Ah receptor may upon translocation to the nucleus undergo a conformational change

(*Ah**) which further stabilizes binding of a charge-transfer type. In this binding mode, the lateral chlorines in TCDD are free to probe the DNA molecular surface for a second receptor (*Rn*) which provides binding pockets that closely match the structure and chemistry of the lateral halogens similar to those found in TBPA binding. In recognizing and binding these pockets the TCDD molecule would probably assume an orientation like that shown in Figure 3 (relative to T4). This would weaken the overall dispersion interactions of TCDD in the two protein complex bringing it more in line with those of T4. It is also quite likely that this cooperative binding event is further stabilized by protein-protein interactions.

The complex resulting from the cooperative binding of these two proteins to TCDD and related structures could activate the subsequent nuclear events leading to a biological response. The cooperative binding event introduces considerable structural specificity to the activation process. If TCDD is viewed as replacing T4 in this process, the normal process may be regulated by the intranuclear metabolic conversion of T4 to T3 which equilibrates in the direction of the cytosol sites to displace more T4 for the nuclear sites to further initiate the biological response. In contrast, TCDD can not be controlled metabolically, i.e., by dehalogenation, and therefore maintains the process in a fully activated state.

Relationship of Mechanism Model to Toxicological Findings

A unifying hypothesis of the mechanism of action of TCDD should not only provide answers to specific toxicological problems but should provide a reasonable biological frame of reference for the diversified effects of TCDD and related compounds. Recent workers (19) have shown that chemical thyroidectomy effectively protects athyroid rats from the toxic effects (body weight loss and mortality) of TCDD whereas nonthyroidectomized-euthyroid and thyroidectomized-T4-maintained-euthyroid rats were not protected. Our cooperative receptor mechanism model predicts that it should be possible to potentiate TCDD toxicity since the *Ah* receptor can function as a site of loss with respect to toxicity (3). We have in fact shown that the thyroid hormones do bind the *Ah* receptor (17) and do potentiate TCDD toxicity as measured in the mouse teratogenicity system (20) and in a bone marrow cell culture system (M. Luster, unpublished observations). Thus the protection derived from thyroidectomy can be possibly explained as a reduction in the potentiation of TCDD toxicity that normally operates in TCDD toxicity with unaltered levels of T3. However, potentiation would be expected to reach a maximum at saturating levels (for the *Ah* receptor) of thyroid hormones.

The striking effects of TCDD on certain organ systems have suggested an endocrine basis, particularly involving glucocorticoids, for its toxicity (7). Glucocor-

ticoids and thyroid hormones are known (21) to have synergistic effects in cell systems in which the glucocorticoid effect appears to be controlled by thyroid hormone; whereas glucocorticoids are not required for thyroid hormone action, they enhance the effect. Consistent with these observations is recent work (22) which suggests that reproducible and stable association of the occupied glucocorticoid receptor to its putative regulatory gene site may require the presence of occupied thyroid hormone receptor which may be adjacent to the glucocorticoid receptor binding site of the gene. Glucocorticoids are also known (23) to induce changes at the level of the nuclear thyroid hormone receptor site. Guinea pigs made toxic with thyroxine show an increased output of glucocorticoids (24). Glucocorticoids can exert dramatic catabolic effects on lymphoid tissue (25). One of the most striking and consistent effects found in TCDD-treated animals is depletion of lymphoid tissues (7), especially the thymus. Thus, varying levels of glucocorticoids in TCDD toxicity may modulate the thyroxine-like effects of TCDD at the level of nuclear receptors.

In terms of the diversified effects of TCDD toxicity, one would expect to find both hypo- and hyperthyroid effects since TCDD does not have the amino acid properties of T4 and, therefore, cannot be a full agonist. The effects of thyroid hormones at the level of nuclear receptors appear to be primarily associated with their halogenated aromatic hydrocarbon character and TCDD is an agonist with respect to these effects. However, the apparent role of thyroxine as a melanin precursor can not be fulfilled by TCDD since it depends on the amino acid properties of thyroxine (26a). Therefore, in TCDD toxicity there would be a lack of thyroxine for this purpose because of its depletion in serum and tissue. Abnormal skin, hair and nail proteins are all signs of hypothyroidism (26b) which are also associated with the toxicity of TCDD and related compounds (1).

Different toxicological endpoints may be mediated by nuclear receptors in various thyroid hormone-responsive organs in which thyroid hormone activity regulates gene expression. Thyroid hormones and nuclear thyroid hormone receptors have been previously implicated in both normal (27) and tumor cell (28) proliferation. Increased protein synthesis and cell proliferation seen in TCDD toxicity can be explained if TCDD acts as a potent and persistent T4 agonist at the level of nuclear receptors. A heuristic model has recently been proposed (29) for the wasting syndrome in TCDD toxicity in which TCDD treatment appears to lower the level of regulated body weight in the animal in a dose-dependent fashion. Hypophagia serves as a secondary response to reduce the animals weight to the lower regulation level determined by the dose of TCDD administered. This set point process could be controlled by the circulating levels of thyroxine. A lethal dose of TCDD functioning as a T4 agonist may initiate this process at the level of tissue receptors resulting in wasting, associated dehydration and death before the lower weight regulation level is

reached. Increased protein synthesis and cell proliferation as shown in the mechanism model (Fig. 4) could be associated with tissue hyperplasia, but it is also possible that the end result of gene expression could give rise to tissue atrophy and hypoplasia (depending on tissue type) where new protein synthesis could generate repressor factors.

Differences in species sensitivities to TCDD toxicity (2) are most likely related to differences in nuclear thyroid hormone binding capacity and pharmacokinetic factors. For example, the guinea pig has about one-third the nuclear thyroid hormone binding capacity of the rat as estimated from serum T4 levels (this is also suggested by preliminary studies involving Scatchard analysis of the thyroxine nuclear receptor in liver) and is unable to eliminate T4 efficiently as can the rat. Because of lower saturation levels for the relevant receptors in addition to the additive effects of TCDD and T4 in occupying these receptors, the guinea pig is a very sensitive animal species to the toxic effects of TCDD. Consistent with this hypothesis is the fact that guinea pig weight loss was used as an early screen for compounds with thyroid hormone activity (30).

We feel that the molecular reactivity characteristics (dispersion interactions involving the ring system and lateral halogens) underlying our mechanistic model for TCDD toxicity have a solid physicochemical basis and can be used in a correlative approach for assessment of activity beyond TCDD and related compounds, perhaps even to structurally unrelated molecules. For example the model predicts that certain highly chlorinated and brominated diphenyl ethers and diphenylmethanes may be toxic. Minimum requirements for toxicity would include two *ortho* halogens (for conformational rigidity), two adjacent lateral halogens (for binding the thyroxine nuclear receptor, R_n) and a sufficient number of halogens in one ring (opposite ring with lateral halogens) to effect a good dispersion, stacking interaction (with the A_h receptor). Also, implicit in our mechanistic model is that the mechanism of TCDD toxicity at the level of nuclear receptors shares common features with the mechanism of thyroid hormone action. Thus our model should provide a new structural and reactivity framework for studying the mechanism of thyroid hormone action.

TCDD as a Thyroxine Agonist and Relationship to Thyroid Hormone Action

Serum thyroid hormones appear to be in equilibrium with tissue nuclear receptor sites (16). In this work, we demonstrate a linear phase for depletion of T4 in rat serum at relatively nonlethal doses of TCDD which when extrapolated to complete T4 depletion may provide an estimation of the lethal dose in this rat species (~ 50 $\mu\text{g/kg}$ body weight). This is undoubtedly associated with the significant enhancement of hepatic uridine diphosphate glucuronyltransferase activity by TCDD in rats previously demonstrated (31) which is near maximal 9

days after TCDD administration. It was further shown at a TCDD dose of 25 $\mu\text{g/kg}$ body weight that there is faster clearance of added T4. Thus serum T4 depletion is reflecting tissue T4 depletion. Even though the ^{125}I -T4 was given orally in this study, it is highly unlikely that these results are due to significant differences in absorption and distribution properties of added T4 because of the small mass (~ 88 ng) of T4 involved. In addition, previous workers (7), using labeled nutrients, found no difference in the absorption and distribution properties of glucose, alanine or oleate in TCDD treated animals versus controls.

Tissue T4 depletion may be correlated with replacement of T4 by TCDD at the level of tissue receptors. In preliminary experiments (data not shown) with rat liver nuclei from rats receiving toxic doses of TCDD, we find an increase in the number of the nuclear receptors for thyroxine consistent with their modulation by thyroid hormones (32). Also in preliminary experiments (data not shown), TCDD and related compounds were shown to compete for specific T4 binding sites in rat liver nuclei in a manner consistent with the predictions using TBPA as a model for the nuclear receptor (5). These observations support the hypothesis that depletion of tissue T4 is a reflection of its replacement by TCDD at the level of tissue receptors in TCDD-treated animals which may be the result of receptor occupancy or alteration by TCDD molecules that persist in the tissues. TCDD toxicity might therefore be in part related to the expression of potent and persistent thyroid hormone activity initiated at the level of the nuclear receptors.

If this is true, we reasoned that it should be possible to demonstrate at subtoxic doses thyroid hormone-like activity for TCDD. We decided to use tadpole growth and development as our model for this, in spite of the fact that previous workers (33) had reported unsuccessful experiments with tadpoles injected with TCDD. Our experiments suggest an initial hyperactive phase in which tadpole length (and growth rate) is increased (detected only in the low dose group) followed by toxicity. Toxicity is expressed as a progressive diminution in size eventually leading to death. Previous workers (34,35) have shown that such rapid weight loss in tadpoles is not related to a decrease in food consumption but rather to a hyperactive state. This reproducible result is in contrast to the no-effect result reported by previous workers (33). Such an initial boost in metabolic rate as seen in this work with TCDD has been well documented for birds receiving various chlorinated hydrocarbon (36) insecticides. There is as yet no indication that such an initial boost in metabolic rate occurs in mammals, but we believe our results with the serum T4 levels in TCDD treated guinea pigs provides an indication of this. It is known (37) that UDP-glucuronyltransferase activity in guinea pigs is not induced on TCDD treatment, in marked contrast to the rat. The elevated serum levels we find in guinea pigs treated with TCDD could be associated with an initial metabolic

boost followed by slow elimination of thyroxine by the transferase system. The effect was already at maximum at 0.5 µg/kg body weight TCDD dose.

Previous workers (38) have studied the effects of TCDD on oxidative phosphorylation rates in isolated rat liver mitochondria. Although no statistically significant effects were seen, there was an indication of an early boost in state 3 respiration which declined and leveled off at later time points. Effects of TCDD on oxidation rates in animals will probably be difficult to demonstrate since any increase will be offset by decreases due to tissue wasting. We feel that these initial boosts in metabolism are the result of increased synthesis of thyroxine derived from the competition of the toxin with thyroxine for the serum hormone binding proteins such as prealbumin or thyroxine binding globulin as proposed (6) for related compounds. However, not all compounds which are capable of binding these serum proteins and stimulating production of thyroxine are capable of binding the nuclear receptors and initiating a response in tissues.

The preliminary finding of earlier and more rapid metamorphosis of tadpoles in the low dose group is consistent with the proposal that TCDD can function as a thyroid hormone agonist and interact directly with relevant tissue receptors. The fact that some tadpoles in this group never undergo metamorphosis and the frogs that are formed die at a faster rate than controls suggests that the dose-response curve for thyroid hormone activity of TCDD is steep and that toxicity is the expression of potent and persistent thyroid hormone activity. Although we made no attempt in this study to measure TCDD levels in tadpole tissue, the persistence of TCDD in animal tissues is well documented (39). The dose-response pattern seen for toxicity in this study along with the very low concentrations of TCDD required to produce effects are consistent with the potency and persistence of TCDD as a thyroxine agonist. TCDD is also a potent embryotoxic agent in rats and mice (40) exhibiting an extremely steep dose-response curve (L. Birnbaum and J. Lamb, personal communication) consistent with its effects on tadpole metamorphosis, a somewhat analogous developmental process.

One would also expect, as in dioxin toxicity, that the Ah receptor interaction may be a necessary but not sufficient condition for hormone activity. Thyroid hormone action may be modulated by a controlled passage across nuclear membranes (41). Nuclear receptor affinity for thyroid hormones and thyroid hormone analogs correlate well with the biological activities of these compounds (26c). However, equal affinity of the nuclear receptor for D and L forms of the hormones, for triiodothyroacetic acid (in spite of lesser biological activity), for reverse triiodothyronine (reported to have little or no biological activity) presents some problems in interpreting the nuclear receptor-binding data. In preliminary work using ¹²⁵I-labeled thyroid hormones and selected analogs in subcellular localization studies, we find that nuclear localization varies in a manner con-

sistent with known biological potencies of the hormones, in spite of equal affinity of some of the hormones for isolated nuclear binding sites. The possibility that hormonal activity is modulated by the Ah receptor is consistent with the observations that T3 binds the Ah receptor with about five to ten times the affinity of T4 (17), potentiates the toxicity of TCDD five times better than T4 (20) and is three to five times more active than T4 as a hormone (42a).

Removal of the ether oxygen to form the linear biphenyl analog of thyroxine is a particularly interesting test model for our mechanism. This analog would be expected to be inactive for the same reasons that *ortho*-substituted polychlorinated biphenyls are nontoxic (43), i.e., both would have weak interactions with the Ah receptor, and thus would have limited access to relevant nuclear binding sites. In limited tests, the biphenyl analogs of the thyroid hormones were shown to be inactive (42b). Other areas of new insight into the mechanism of thyroid hormone action predicted by our model for which experimental support already exists include the multiple component nature (44) of the nuclear binding events and that T4 has intrinsic hormonal activity and is not merely the prohormone of T3 (45). Since T3 can potentiate the toxic effects of TCDD and since TCDD can replace T4 in biological systems, it is possible that T3 is a natural potentiator of T4 activity. In preliminary studies (M. Luster, unpublished observations), we have in fact qualitatively reproduced TCDD toxicity by using combinations of T3 and T4 while either hormone alone was ineffective. This result suggests that combinations of T3 and T4 are much more effective in producing thyroid hormone activity than either hormone alone. The fact that T4 is selectively depleted in TCDD toxicity is also consistent with it being the controlling form of the hormone.

Finally, this work suggests a logical role for reverse T3 (rT3) in thyroid hormone action as a natural intranuclear antagonist of T4. Reverse T3 would be expected to bind weakly to the Ah receptor and have only limited access to nuclear binding sites because of conformational flexibility (12) and the presence of only one iodine atom in the nonphenolic ring. This is supported by preliminary findings (data not shown) that reverse T3 shows little accumulation in nuclear fractions in rat liver cells. Its presence in the nucleus is probably controlled by nuclear metabolism of T4. The intranuclear formation of rT3 is also consistent with the observations that rT3 binds to solubilized nuclear receptors (46) for thyroxine but fails to bind when intact nuclei are used (47). Therefore, rT3 formed in the nucleus can bind the nuclear T4 receptors (although with a lower affinity than T3 or T4) but can not simultaneously bind both the nuclear receptor and the cytosolic receptor to effect a biological response as illustrated in Figure 4 for TCDD. This model for antagonism differs from the one for potentiation where the potentiating chemical has a preference for the cytosolic receptors and increases the population of active molecules available to nuclear receptors. In pre-

liminary work, we have demonstrated antagonism of TCDD toxicity by structures related to dioxin as well as thyroxine in a bone marrow cell culture system. These antagonists are capable of binding either the Ah receptor or the thyroxine nuclear receptor alone but can not simultaneously bind both receptors with different structural parts (as shown for TCDD in Fig. 4). Thus this receptor model is also pointing to new directions in the search for the highly elusive competitive inhibitor of the thyroid hormones (antithyroid drugs).

Conclusions

The resistance to metabolism and persistence of TCDD in animal tissue are well documented (39). Because of this persistence in tissue and common molecular reactivity properties, TCDD can replace thyroxine in the tissues at the level of nuclear receptors and act as a highly active but metabolically uncontrollable thyroid hormone analog. The most serious end result is a runaway catabolism (probably to meet an increased requirement for metabolic energy) resulting in a wasting syndrome and associated dehydration ultimately leading to death (at lethal doses) which resembles in many ways the toxicity produced by thyroxine itself (25). The thyroid gland would be isolated in a hyperactive state (8), since it is reacting to what it detects is a reduction of thyroxine (local T4 metabolism is primary source of pituitary T3).

Effects on tadpole growth and development provide strong supporting evidence that the mechanism of dioxin toxicity is the mechanism of thyroid hormone action. This is also supported by preliminary studies with the nuclear receptor for thyroxine. TCDD may be a very unusual thyroid hormone analog in that it is capable of binding both T3 and T4 receptors with high affinity. In terms of thyroid hormone activity, the TCDD molecule accomplishes in one structure what normally requires two different structures (T3 and T4) working in cooperation through potentiation and metabolic regulation to maintain the appropriate levels of nuclear T4. The environmental health concern over TCDD and related compounds would be clearly justified if these compounds can function as potent and persistent thyroxine agonists and produce a number of health effects associated with hypo- and hyperthyroidism.

A molecular mechanism is proposed involving a two protein-receptor model in which the planar aromatic system controls the initial receptor binding in cytosol and halogen substituents control subsequent nuclear events. Although a complete delineation of the mechanism at the molecular level has not been done, the proposed mechanistic model based on molecular reactivity characteristics has brought immediate insight to an understanding of the diversified effects of TCDD and related compound toxicity and certain thyroid hormone action. In addition, the mechanistic model also permits predictions to be made with regard to the toxicity and thyroid hormone activity (agonists, antagonists, etc.) of

untested compounds. In fact, the two separate lines of research are in many ways mutually parallel and reinforcing.

Finally, the work has suggested a general mechanism for hormone action based on metabolically regulated differential and cooperative receptor binding events in cellular compartments which can explain agonism, antagonism and potentiation within the framework of receptor occupancy theory (48). In this general mechanism, a metabolite (like T3) of the active hormone (T4) would bind cytosol receptors with somewhat greater affinity than the active hormone itself. The metabolite can displace the active hormone from the cytosolic receptor freeing the hormone for binding to nuclear sites on chromatin to which it binds with greater affinity than the metabolite. This push to nuclear binding of the active hormone is enhanced as the concentration of metabolite increases by intranuclear conversion of the hormone to the metabolite. The active hormone is maintained at a fairly constant level in body fluids and tissues by the appropriate organ systems (pituitary/thyroid axis). The molecular reactivity properties of the hormone can be viewed as a steering mechanism for bringing together the two proteins on chromatin and initiating the biological response. Once the hormone has accomplished this task, it may be metabolized with a different biological half-life than that of the activated protein complex itself.

This process requires binding to two different proteins by two different chemical mechanisms in order to initiate a response, thereby establishing structural specificity. The necessary concentration gradient to regulate the process is established by differential binding of the active hormone and its metabolite to the two proteins. This would have its analogy in steroid hormone action where testosterone is known (49) to be an active hormone and is also converted into dihydrotestosterone, which is twice as potent possibly because it is a better potentiator of testosterone activity than is testosterone itself. This points to new directions in studies of hormone receptor biochemistry and drug design and suggests that toxicity studies involving chemical combinations will produce interesting results.

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